EVALUATION OF HOT WATER WASH PARAMETERS TO ACHIEVE MAXIMUM EFFECTIVENESS IN REDUCING LEVELS OF *SALMONELLA* TYPHIMURIUM, *ESCHERICHIA COLI* O157:H7 AND COLIFORMS/ *ESCHERICHIA COLI* ON BEEF CARCASS SURFACES

A Thesis

by

MELISSA ANN DAVIDSON

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2010

Major Subject: Food Science and Technology

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Approved by:

Co-Chairs of Committee,	Kerri B. Harris
	Jeffrey W. Savell
Committee Member,	Alejandro Castillo
Chair of Food Science and Technology Faculty,	Jimmy Keeton

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ABSTRACT

Evaluation of Hot Water Wash Parameters to Achieve Maximum Effectiveness in Reducing Levels of *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and Coliforms/*Escherichia coli* on Beef Carcass Surfaces. (May 2010)

Melissa Ann Davidson, B.S., Texas A&M University;

M.S., University of Arkansas

Co-Chairs of Advisory Committee: Dr. Kerri B. Harris
Dr. Jeffrey W. Savell

This study measured and compared different temperatures and dwell times of hot water treatment on the reduction of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on beef carcass surfaces. Two different types of beef surfaces, lean and fat, were inoculated with a fecal slurry containing *E. coli* O157:H7 and *S.* Typhimurium at ca. 7-log CFU/g, washed to remove gross fecal matter, and rinsed with hot water between 66 and 82ºC (150 to 180ºF water) for either 5, 10, or 15 s. There were no differences ($P > 0.05$) in the log reductions of *S.* Typhimurium and *E. coli* O157:H7 on the lean surfaces for all three temperature treatments (66, 74, and 82°C). Although the 15 s treatment resulted in a numerically higher log reduction than the other treatments, each of the times resulted in at least a 1 log reduction of both *S.* Typhimurium and *E. coli* O157:H7 for lean surfaces. For the fat surfaces, all time treatments for the 82°C and the 10 and 15 s treatments for the 74°C resulted in the highest log reduction for *S.* Typhimurium. The 5 and 10 s dwell times for treatments at 66°C and the 5 s dwell time at 74°C resulted in the lowest log reduction of *S.* Typhimurium and *E. coli* O157:H7. For *E. coli* O157:H7 all

temperature and time treatments resulted in at least a 1 log reduction for the fat surfaces of the outside round. Therefore, hot water treatment is a proven method for reducing both coliforms and pathogenic bacteria.

DEDICATION

I dedicate this thesis to my parents Dennis and Georgia Davidson. Without their love and constant support, this degree would not be possible. I love you very much.

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NOMENCLATURE

C	Celsius
CFU	Colony Forming Unit
cm	Centimeter
h	Hour
L	Liter
min	Minute
ml	Milliliter
kgf/mm2	Kilogram-force per square millimeter
s	Second

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CHAPTER I

INTRODUCTION

The beef industry continues to battle foodborne pathogens every day. In 1999, it was found that pathogens such as *S.* Typhimurium and *E. coli* O157:H7, which can be found on raw beef, have been associated with causing approximately 6 million illnesses and 9,000 deaths each year *(24).* Although in 2004 that number had started to decrease, the beef industry still faces pathogen control every day. There are various interventions that the industry employs such as hide interventions, steam vacuuming, organic acids, trimming, and hot water usage to decrease pathogen presence. These have proven to decrease pathogen numbers when used properly.

Hot water has been shown to be an effective microbial intervention for beef products standing alone or in conjunction with organic acids *(8, 9, 13, 14, 22).* It has been reported that the surface type, fat or lean, can have an impact on the microbial reduction when subjected to the same water wash treatment *(9).* Temperatures and dwell times also can vary depending on the facility's ability to maintain the hot water temperature, line speed, and safety to workers.

The objectives of this project were to determine the potential of hot water applied over varying exposure times and temperatures to reduce the levels of *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and coliforms/*Escherichia coli* on beef carcass surfaces, and to evaluate the effect of various exposure times and temperatures of hot water on meat surface type (fat and lean).

This thesis follows the style of the *Journal of Food Protection*.

CHAPTER II

LITERATURE REVIEW

Generic *Escherichia coli*

Generic *Escherichia coli*, once known as *Bacterium coli commune*, is a member from the family Enterobacteriaceae. Other members of the Enterobacteriaceae family include many other known enteric pathogens such as *Salmonella* spp., *Shigella* spp, and *Yersinia* spp, all of which could be pathogenic to humans. *Escherichia coli* is a gram negative, rod shaped, facultative anaerobe, oxidase-negative, which ferments glucose with the production of gas and acid *(19)*. *Escherichia coli* is a predominant part of intestinal microflora for various species, which includes cattle, sheep, dogs, and humans. Humans have over 400 beneficial bacteria species including *Escherichia coli* in the intestinal tract mostly residing in the ileum and lower bowel *(18)*. *E. coli* is approximately 0.1% of the total normal microflora bacteria within an adult's intestines (on a Western diet), and, in a newborn infant's intestines, *E. coli*, along with lactobacilli and enterococci, represent the most abundant bacterial flora *(6)*. *Escherichia coli* is an established part of the normal microflora which aids in the digestion of food, synthesizing vitamin K and B complex, and preventing the chance for pathogenic bacteria to colonize the intestine by suppressing their growth *(18, 43)*.

From humans to chickens, *E. coli* is a symbiotic bacterium, able to inhibit pathogenic bacteria from becoming established in the intestine by means of out-competing for space and/or nutrients. Gorbach *(18)* illustrates (Figure 1) that most of the beneficial bacteria reside in the ileum and large bowel in humans.

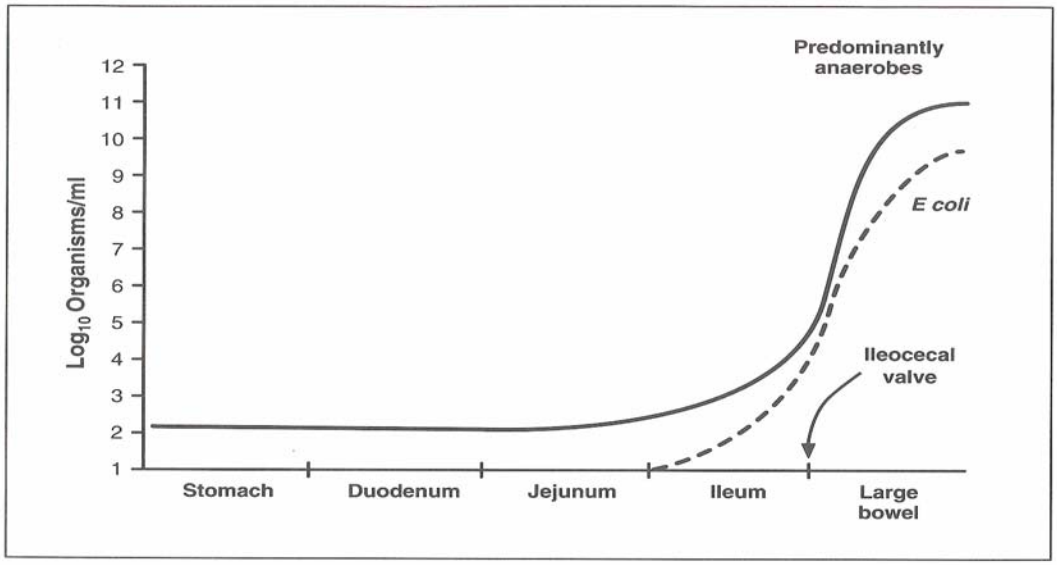

Figure 1. Concentration of the bacterial flora in regions of the gastrointestinal tract.

When the generic non-pathogenic *E. coli* reaches its optimum growth environment, the replication time for one cycle is as little as 20 min *(29)*. This somewhat prophylactic use is also known as probiotics, or Competitive Exclusion (CE) *(36)*. It is able to outgrow other bacteria thus hindering other bacteria from growing in the crypts of the intestine.

Pathogenic *Escherichia coli*

Escherichia coli O157:H7 is a part of the *E. coli* family, but is known to be pathogenic to humans. Its sources are usually from eating raw beef. It first enters the body via food eaten by an individual. Once inside the body, the microorganism can become established and begin to cause discomfort harm to the individual who consumed it.

Because of the ability of *E. coli* O157:H7 to replicate quickly, one or two cells in food could easily turn into an infectious dose *(27)*. After surviving the harsh stomach conditions in a human, Enterohemorrhagic *E. coli* (EHEC) attaches to the host's epithelial cells in one of two ways. It can form either tight clusters or microcolonies forming an even, single layer over the epithelial *(32)*. The four mechanisms that cause gastroenteritis in humans are: adherence to mucosa cells, invasion of mucosa cells, disruption of the microvillus brush border, and the toxin release *(43)*. Once the toxins are released by the *E. coli* O157:H7 bacterium, damage occurs to the intestinal epithelium *(43)*. When the verotoxins are absorbed through the intestinal tract, complications occur in other organs in the body.

The pathogenic categories of *E. coli* are broken into five main categories: Enterotoxigenic *E. coli* (ETEC), Enteroaggregative *E. coli* (EAEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), and Enterohemorrhagic *E. coli* (EHEC). Figure 2 from Evens *(15)* illustrates the relationship of some of these bacteria.

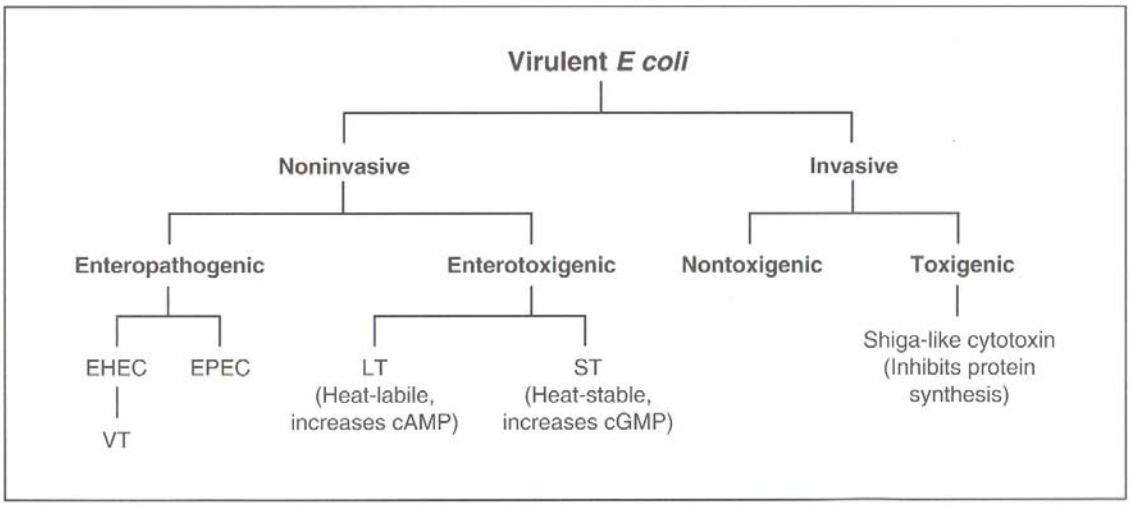

Figure 2. Virulence mechanisms of *E coli*.

Enterohemorrhagic *E. coli* (EHEC), or Vero Cytotoxin-Producing *E. coli* (VTEC) and Shiga-toxin Producing *E. coli* (STEC) are the last of the *E. coli* segments to date. This set comprises unusual bacteria that can produce hemolytic uremic syndrome (HUS) and/or thrombotic thrombocytopenic purpura (TTP). *E. coli* O157:H7 is included in this category.

***Escherichia coli* O157:H7**

Escherichia coli O157:H7 is a facultative anaerobe that is gram negative and produces a Shigella-like toxin (S*lt*). It is thought that *E. coli* O157:H7 evolved from *E. coli* O55:H7, a pathogen associated with infant diarrhea worldwide *(3)*. Although the *E. coli* O55:H7 bacterium possessed the mechanism for adherence to the mucosal cells, it did not produce any toxin. When it acquired the S*lt* genes from Shigella through horizontal gene transfer, O157:H7 was created*(3)*.

There are two types of shiga toxins: Slt1 and Slt2. Toxin typing can help identify *E. coli* O157:H7. Human isolates that produce Slt1 and Slt2 or only produce Slt2 are common, whereas those that only produce Slt1 are uncommon *(19).* Although they are called shiga-like toxins, Slt1 shares very similar gene coding with that of *Shigella dysenteriae* but Slt2 only shares 58% overall homology with that of Slt1 *(19)*. This explains why Slt1 can be neutralized with anti-Shiga toxin and Slt2 cannot *(19).*

E. coli O157:H7's average onset is approximately 3 to 4 days. However, it has been known to have short incubation times of 1 to 2 days, or as long as 5 to 8 days. The complications begin with watery-like diarrhea, abdominal cramps, and mild, to no, fever. Within days, the diarrhea was come bloody with increased cramping which normally lasts from 4 to 10 days *(11).* With severe O157:H7 cases, there may be all blood and no stool.

Most of *E. coli* O157:H7 infections occur in the younger population (less than 5 years of age) and the elderly (over 65 years of age) *(1).* People who are immuno-compromised such as people who are taking, chemotherapy or have HIV, are not considered to have a special risk factor and do not have an increased probability of contracting *E. coli* O157:H7 compared to a normal, healthy middle-aged individual.

Most of the people infected with *E. coli* O157:H7 will recover completely without the use of antibiotics, but 10% of the patients, mostly the young and the elderly, acquire hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura (TTP). Although in most bacterial infection cases antibiotics will help the situation, however, with *E. coli*, antibiotics will only increase the chance of kidney complications *(11).*

Although almost all strains of *E. coli* O157:H7 are susceptible to ampicillin, trimethoprim-sulfamethoxazole, tetracycline and quinolones, they are resistant to many other antibiotics such as erythromycin, metronidazole, and vancomycin *(1)*. Studies have shown that the use of such antibiotics, while they may kill the bacteria, could leave more damage in their wake such as an increasing HUS development because of the toxin released by the dead cells *(1)*.

Due to the severity of *Escherichia coli* O157:H7 and the damage it causes, it is recognized as a reportable organism, and, as such, confirmed stool specimen cases should be reported to the county and state health departments where the case occurs *(11)*.

Hemolytic uremic syndrome (HUS)

Hemolytic uremic syndrome (HUS) is caused by the *E. coli* O157:H7 cell being lysed, (cut) or killed. When this happens the cell releases toxins in the blood stream that causes the red blood cells in the blood stream to lyse.

According to the CDC, 20,000 people are infected with *E. coli* O157:H7, and of those 20,000, approximately 250 will die, mostly from HUS and other complications *(33)*. One third of the people who develop HUS will have permanent kidney damage, and another 8% will have other lifelong complications such as hypertension *(11, 33)*. In the United States, Hemolytic Uremic Syndrome (HUS) has become the leading cause of pediatric renal failure requiring a kidney transplant *(33)*.

Thrombotic thrombocytopenic purpura (TTP)

Thrombotic thrombocytopenic purpura is a rare, yet often fatal condition in which a blood clot gets in the brain and causes some symptoms such as “neuologic abnormalities, microangiopathic hemolytic anemia, thrombocytopenia, and potentially

renal failure" *(11)*. Most patients who develop this condition have had an *E. coli* O157:H7 infection in the past. In fact, most patients who have had TTP have the same pathologic features, yet do not develop gastrointestinal infections. Although neurologic manifestations and fever are usually the main signs of TTP, HUS rarely has the same symptoms; however, it is possible.

Salmonella

Salmonellosis is reported to be the most common foodborne illness in the US *(9)*. Although there is a reported 47,500 cases reported annually in the US, because of the unreported cases, the actual number is estimated to well over 2,000,000 a year *(28)*. It can be found in various food sources ranging from poultry, eggs, lettuce, ground beef, veal, turkey, swine, pork, and peanut butter *(21)*.

Salmonellosis develops usually 12-14 hours after consumption of ingestion of the food source depending on the person. Symptoms can include vomiting, nausea, abdominal pain, chills, headache, and diarrhea lasting 2-3 days *(21, 28)*. Although the mortality is usually low (4.1%) it ranges in people from under a year (5.8%), between 1-50 years old (2%), and over 50 years of age (15%) *(21)*. The infectious dose ranges from 10^5-10^6/g have been suggested to cause salmonellosis *(21)*. This dose again, will depend on the individuals' health, age, and ability to fight off an infection. If these could be a factor, the infective dose could be lowered to 10^3-10^4/g for that individual.

Most people recover from *Salmonella* without any treatment. Although there is no known sequelle to *Salmonella*, this pathogen can reside in macrophages and invade the body destroying the Peyer's patches, part of the mucosal-associated lymphoid tissue that samples the small intestine for foreign antigens *(28)*. This is a problem in the beef

industry. Although most of the lymph nodes are discarded during evisceration, there are some lymph nodes located in the fatty tissue of the beef carcass that does not get trimmed out and can lead to contamination if the infection is bad enough *(2)*.

***Escherichia coli* O157:H7 and the beef industry**

E. coli O157:H7 was coined the "hamburger disease" in 1993 because of an outbreak from the Jack-in-the-Box chain of hamburgers *(5, 22)*. This outbreak led to the deaths of three children and hundreds of other people becoming ill *(24)* in as many as four states *(11)*. Because of the damage this pathogen caused and the publicity it received, the United States Department of Agriculture Food Safety Inspection Service (USDA, FSIS) changed the way meat and poultry establishments were inspected from then on. The Pathogen Reduction /Hazard Analysis and Critical Control Points (HACCP) regulation was finalized in 1996.

Between the years of 1982 and 1994, 50% of *E. coli* O157:H7 infections were due to hamburgers *(5)*. The U. S. beef industry has incurred significant cost due to *E. coli* O157:H7 *(24)*. In the past 10 years, the beef industry has lost $2.7 billion due to recalls, increased operating costs, government and industry research, etc., and a decrease in demand for beef, which cost the beef industry $1.6 billion alone *(24)*.

The first recorded incident of O157:H7 occurred in 1975 when a woman having bloody diarrhea was admitted to a hospital. Her blood tested positive for antibodies to the O157 and H7 antigens, specific components of *E. coli* O157:H7 *(26, 41)*. However, because she was the only one who was sick, it was not considered an outbreak of the organism, and the public was not made aware of the bacterial infection *(41)*. The Centers

for Disease Control (CDC) kept an isolate in reference in case it was to appear in later cases *(41)*.

Because of the seriousness of this pathogen and the illness it can cause, processors must understand how to prevent and control *E. coli* O157:H7 *(17)*. Since the 1993 outbreak, *E. coli* O157:H7 has been a growing problem with the beef industry. Today, people are more aware of the serious issues with *E. coli* O157:H7, and the beef industry continues to seek ways to minimize the risk associated with beef.

Frequency of *Salmonella* and *Escherichia coli* on meat

In September 1999, FSIS began using a method for analyzing samples of products that may contain O157:H7 that was four times more sensitive than the previous tests being used *(16)*. When testing this new method using the same product, 40% (21 out of 53) of the samples tested positive for *E. coli* O157:H7 with the new system *(16)*. With the old system (used since 1994), the samples tested negative with lower levels of *E. coli* O157:H7 *(16)*. This suggests that the tests using the old system were not as sensitive in detecting lower levels of *E. coli* O157:H7 and this pathogen are more prevalent than once was thought. These tests were made because of the severity of the damage that *E. coli* O157:H7 caused due to its high virulence and relatively low doses in which it could cause such damage. Experts have stated the infective doses can range from 10-100 organisms, depending on the age and health of the individual *(16, 35)*, but Rhee et al. said the infectious dose might be as low as two *(37)*.

Many experiments have been performed on the bovine species to determine where in the gastrointestinal tract the bacteria are present. Researchers have found the presence of O157:H7 in the colon region of the cattle tested, as well as in other parts of the animal

(3). However, Brown et al. *(6)* reported the fore stomach as the prime location for proliferation and localization. Buchko et al. *(7)* found the cecum and rectum were sites of colonization.

Cattle hides have been found to be a source for *E. coli* O157:H7. It has been determined that during processing, the primary source of contamination is the transfer of matter from the animal's hide to the carcass *(31)*. Particular regions, which have tested positive, are the back and tail areas. This is probably due to the fecal contamination from the feces on the tail, which, in turn, swipes the back region to keep flies away. When the hide is removed during processing, the bacteria can contaminate the carcass. Because cattle are asymptomatic, presence of this particular organism will not show through mortality or morbidity *(3)*.

Some variables that contribute to the prevalence of this organism include the season, frequency, timing between testing, and the geographic location of herds *(3)*. Seasons tend to influence the prevalence of the O157:H7 bacterium. Belongia at al. *(4)* conducted an experiment concerning rural and non-rural resident children and their antibody levels for *E. coli* O157:H7. Belongia et al. *(4)* found that rural children had a higher antibody level for O157:H7 than non-rural children. Belongia et al. *(4)* also discovered that the occurrences of these IgM antibodies in children were higher in the summer months, July through September, and lower in the cooler months of January through March. This coincides with the outbreaks in cattle *(3, 4)*. Buchko et al. *(7)* performed several studies that concluded the peak fecal shedding of *E. coli* O157:H7 was during the spring and summer months.

Salmonella can be a seasonal pathogen like *E. coli*. The fluctuation can range anywhere from a 2.1% fecal prevalence to a 9.1% within a year's time *(8)*. This also is parallel with the carcass contamination, immediately after hide removal, having reported numbers ranging from 3%-24.9% within a year *(8)*. This shows us that even in *Salmonella's* low season it is still able to contaminate around 3% of the carcasses pre-evisceration.

Cost to producers

The cost estimated stems not only from recalls, but also to the decrease in confidence the consumers have in the safety of eating beef. It is said that recalls themselves might have cost the industry $1.6 billion of the $2.7 billion total *(24)*. Due to the bad publicity *E. coli* O157:H7 has received in past years; many consumers are concerned about eating undercooked hamburger because of the fear of being infected with this pathogen *(35)*. Not only did it change the minds of the consumers, but it also changed the way the government looked at the way meat and poultry were inspected for pathogens.

After the 1993, outbreak mandatory Hazard Analysis and Critical Control Point (HACCP) was implemented in all meat and poultry plants. Hazard Analysis and Critical Control Point made the plants identify all potential biological, chemical or physical hazards in the process *(34)*. The Pathogen Reduction/ HACCP Rule in 1996 was a direct consequence in the identification and ongoing presence of *E. coli* O157:H7 in ground beef *(24)*. The Food Safety and Inspection Service was originally estimated to be a fraction of a cent per pound by the government in order to be implemented; however,

university studies proved otherwise and estimate costs to be as high as 17 cents for beef *(24).*

Other interventions

Other interventions have been used in plants to retard or inhibit growth of microorganisms. It has been proven that in large US processing plants when 53.9% of beef carcasses were positive with at least one strain of non-O157 STEC prior to evisceration can be reduced to 8.3% with various intervention strategies *(25).*

Chemical dehairing works using sodium sulfide to dissolve the hair from the hide then neutralities it with hydrogen peroxide *(9).* Although this process may raise some questions about its effectiveness, in combination with other interventions could decrease the pathogen presence.

Spray wash using ambient temperature can remove 1 log/CFU of aerobic bacteria per cm^2 *(10)*. Using an organic acid can also aid in retarding the growth of or eliminating the presence of pathogens of concern. Lactic acid is commonly used in commercial practice and applied prior to placing in the hot box*(25).* The effectiveness of these acids can lose their efficacy if they are applied to the surface after chilling *(20)*.

How heat processing affects the cell

Heat is often a way producers try to control potential pathogens on meat surfaces. High temperature water washes have been proven to reduce pathogen presents.

Although high temperatures can affect the *Escherichia coli* O157:H7 bacteria, no one single event of heating is responsible for the effects of the cell because all of the components of the cell are affected. *Escherichia coli* O157:H7, like most non-sporulating bacteria, are inactivated or killed, at ≥50°C.

The cell wall and outer membrane protect the bacterium from outside harm. If heated, morphological and structural changes to the wall and membrane occur which can alter the permeability of the cell, releasing essential periplasmic proteins, UV-absorbing materials, and cations, and letting in otherwise impermeable hydrophobic antibiotics, thus injuring the cell *(39, 42)*. Russell and Harries *(39)* discovered that temperatures ranging from 50°C to 60°C on *E. coli* O157:H7 in suspensions increased the leakage of the cells with an increase of time and temperature. Although heat can cause damage to the cell wall, it is probably not significant in the role of the cell's destruction or inactivation.

The next layer known as the cytoplasmic (inner) membrane is located beneath the cell wall. It is responsible for controlling the entry of solutes into and out of the interior of the cell. In particular, the membrane has many functions that are essential for cell growth and survival. One in particular is the maintenance of the cell's integrity *(23)*. Damaging this membrane can cause serious injury to the cell because the cell cannot regulate any activity going into or leaving the cell *(38)*. However, Welker *(44)* states there is little direct evidence that any membrane damage is important in the thermal injury to the cell. Although the cell's membrane damage is not the major cellular site for destruction, it is important for the cell to repair the damage in order for it to survive *(23)*.

Deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are next in line that could be affected by heat. Mild heating can degrade RNA, which precedes the loss of viability. However, it is not solely responsible for cell death *(38)*. Heat application to DNA could possibly hold the key to inactivation. If the entire DNA is degraded or destroyed, then the cell will not survive *(38)*. The loss of viability of cells is a correlation between mild heat and the introduction of single-strand breaks in the cell's DNA *(44)*.

However, if a functional fraction of the DNA structure is intact, it can reconstitute the other parts of the double stranded or single stranded breaks of the DNA thus enabling the cell to repair itself *(38).* Russell *(38)* stated that pyruvate present in the medium was a key part in the recovery of *E. coli* O157:H7.

Another key part in the inactivation of *E. coli* O157:H7 is protein denaturation and enzyme inactivation. Structural and functional proteins are prime targets for heat inactivation *(38).* Protein denaturation is the disruption of an original, natural, largely hydrogen-bonded, and complex structure *(38).* Russell has overwhelming evidence that protein denaturation correlates with death rates in mesophilic bacteria.

Although the destruction of the pathogenic bacteria may prove to be beneficial to humans, it is also beneficial to other heat injured survivor cells. Material leaked from dead cells can act as a nutrient to survivors and could possibly aid in the recovery and survival of others *(38).*

How can water wash impact the industry

Because heat can have a great impact on a bacterial cell when the conditions are right, the hot water wash can be a tool used in the processing plant that will help keep the bacterial load down. These two pathogens are chosen because they represent the top human pathogens in meat *(12).* Although hot water washes experiments have either observed combination or “multiple hurdle” interventions *(9),* and have not looked at the hot water wash as the only source of intervention. This experiment was designed to look at that and help industry better understand about how these pathogens react to different surface types and different temperatures.

CHAPTER III

MATERIALS AND METHODS

Strains and inoculum preparation

Rifampicin-resistant strains were derived from parent strains of *S.* Typhimurium ATCC 13311 and *E. coli* O157:H7 (Phillip I. Tarr strain) were used as marker organisms to inoculate the fecal slurry and beef carcass surfaces to be treated in this study. Growth curves, heat resistance, and acid sensitivity of the mutant strains were previously determined to be virtually indistinguishable from the parent strains *(20)*.

The selected rifampicin-resistant cultures were maintained on tryptic soy agar (TSA; Becton Dickinson, Sparks, MD) slants at 4°C. Before inoculation into feces, these cultures were transferred into tryptic soy broth (TSB; Becton Dickinson, Sparks, MD) and incubated at 37°C for 18 h. A bacterial cocktail was prepared by mixing equal volumes of each culture for addition into fecal slurries and subsequent inoculation onto meat surfaces. Fecal slurries were prepared from bovine feces collected from the Texas A&M University Beef Center, College Station, Texas. Ten-gram portions of feces were weighed out into individual stomacher bags for subsequent inoculation. Ten ml of the bacterial cocktail were added to each bag and hand-kneaded for 1 minute to ensure homogeneous distribution of the marker organisms. The inoculum was used within 6 h after preparation. Preliminary investigations indicated the marker organisms would not increase in number for up to 6 h when held at ambient temperatures. Additional portions of fecal slurry were prepared with 10 ml of 0.1% peptone water for inoculation of controls to evaluate reductions in naturally-occurring *E. coli* and total coliforms. A

portion of uninoculated feces and the inoculated fecal suspension was assayed each test day to confirm the amount of background *E. coli*, total coliforms, and the number of marker organisms per gram.

Inoculation

Hot-boned inside (IR) and outside rounds (OR), similar to Institutional Meat Purchase Specifications #168 and #170, respectively, were obtained from cattle (n=14) harvested at the Rosenthal Meat Science and Technology Center (RMSTC), Texas A&M University *(30)*. The cattle were typical of those entering the United States meat supply and were harvested following typical procedures in an inspected establishment. Exterior surfaces from each cut (representing subcutaneous fat for the OR and lean for the IR) were used for the decontamination trials. These exterior surface types represent types of carcass surfaces commonly trimmed when fecal contamination occurs during harvest and dressing. The cuts were removed from the carcass just subsequent to splitting and before washing. After removal, the hot subprimal cuts were covered with shrouds, placed in insulated containers, and transported to the Food Microbiology Laboratory (located in the adjacent building). Immediately upon arrival, the carcass surface region was inoculated with the previously prepared fecal slurry, containing either inoculated or non-inoculated feces, using a sterile stainless-steel spatula. Inside round pieces were trimmed of excess fat as needed, using a sterile knife, to expose a greater lean surface area for treatment. The concentration of each pathogen, coliforms and *E. coli* on the meat surface was approximately 6 and 5 log CFU/ cm^2, respectively.

Sampling and microbiological analysis

Thirty min after inoculation, two composite samples were collected from the surface to obtain background and initial inoculum levels. A composite sample was collected consisting of two 10-cm^2 pieces excised from the meat surface with a sterile surgical blade and sterile forceps. Both pieces were transferred to a stomacher bag with 99 ml of sterile 0.1% peptone water and pummeled in a stomacher (A.J. Seward, London, UK) for 1 min. Rifampicin-resistant pathogens were enumerated by plating appropriate dilutions of the composite samples on pre-poured and dried lactose-sulfitephenol red-rifampicin (LSPR) agar *(10)*. Plates were incubated at 35ºC for 24 h before colonies were counted. Total coliform and *E. coli* counts were determined from rounds inoculated with feces containing naturally-occurring background flora. Appropriate dilutions were plated on Petrifilm *E. coli*/coliform count plates (3M, St. Paul, MN) and incubated at 35ºC for 24 h.

Treatments

After inoculation with the fecal suspension, a water wash was applied to each inside and outside round to remove gross fecal contamination. The initial water wash consisted of ~1.5 liters of water and was applied at 25ºC and 0.01 kgf/mm^2 for 90 s using a polyethylene hand sprayer (Ortho Heavy Duty Sprayer, Fountainhead Group, Inc., Newark Mills, NY). Following each initial water wash, the inside or outside round were sectioned into four pieces using a flame-sterilized knife. Following division of the initial round into subsample pieces, composite samples consisting of two 10-cm^2 pieces excised with a sterile surgical blade and sterile forceps were collected from the carcass surfaces to establish initial background counts before treatment. Each piece was assigned randomly

to each treatment. Treatments evaluated were: a) hot water at 66 ± 1ºC for 5, 10 and 15 s; b) hot water at 74 ± 1ºC for 5, 10 and 15 s; c) 82 ± 1ºC for 5, 10 and 15 s. The treatments were applied in a model spray cabinet (Chad Company, Lenexa, KS) using a flat-spray nozzle (50/50) at a pressure of 0.02 kgf/mm2 from a average distance of approximately 16.5 cm. The water was heated in a hot water tank (Chad Company, Lenexa, KS). The temperature of the water at the source and at the nozzle opening was measured using type K thermocouples connected to a total range digital thermometer (VWR International, West Chester, PA). The temperature of the carcass surface during the treatment was measured by placing type K thermocouples threaded through the meat to lay on the meat surface and connected to the same type of thermometer previously described. In addition, temperature indicator labels (Omega, Stamford, CT; Palmer-Wahl, Asheville, NC) and an infrared thermometer (VWR International, West Chester, PA) were assigned randomly to pieces for supplementary monitoring of meat surface temperatures during treatment. The experiment was repeated three times. Following each treatment, two composite samples as previously described were collected from the treated surface. Both pieces were transferred to a stomacher bag with 99 ml of sterile 0.1% peptone water and pummeled in a stomacher (A.J. Seward, London, UK) for 1 min. Appropriate decimal dilutions were plated on pre-poured and dried LSPR agar plates for recovery and enumeration of rifampicin resistant pathogens (*E. coli* O157:H7 and *S.* Typhimurium). All plates were incubated at 35ºC for 24 h before colonies were counted.

Color evaluation

Carcass surface regions were evaluated visually for initial color and changes in color before treatment, after treatment, and after 24 h storage at refrigeration (4 ± 1ºC) temperature by a trained evaluator using meat color evaluation scales adapted from Hunt et al.

Statistical analysis

Plate counts for each organism were converted to log CFU per ml or square centimeter before analysis. Log reductions (CFU/cm^2) of indicator organisms and pathogens were calculated by subtracting the log counts (CFU/cm^2) obtained after hot water treatment from the log count (CFU/cm^2) obtained after water were before any treatment was applied. Data were analyzed using PROC GLM of SAS as a randomized incomplete block design with temperature and time as the main effects and processing day as the block. Temperature by time interaction was included in the model. Least squares means were calculated and where Analysis of Variance indicates significance ($P < 0.05$), differences of least squares means were determined using the pdiff procedure of SAS.

CHAPTER IV

RESULTS

The results from this study support the findings of previous research that demonstrated hot water treatments effectively reduce microbes on beef carcass surfaces. One objective of the project was to determine if there were differences existed in log reductions between application times of 5, 10, and 15 s for three different hot water temperatures (66 ,74 , and 82°C). The second objective was to observe the hot water temperatures (66, 74, and 82°C) on reduction potential.

Table 1 provides the log reductions of *S.* Typhimurium and *E. coli* O157:H7 on the lean surfaces of the beef inside round. Even though statistical differences occurred, overall, no differences ($P > 0.05$) existed in log reductions of *S.* Typhimurium and *E. coli* O157:H7 on the inside round regardless of temperature (66, 74, and 82°C) (data not shown). Differences between 5 and 15 s was observed for overall treatments, regardless of the temperature for both pathogens. Applying the hot water treatment for ten seconds proved not to have a statistical difference from either of the two times.

Table 2 shows the interaction of time and temperature on log reduction of pathogens on fat surfaces of beef outside rounds. The sixty-six degree treatment proved to be the only temperature that showed a difference between 5 and 15 s, with 10 s showing no statistical difference, while other temperatures showed no statistical difference between the 5 and 15 s treatments for either pathogen. The time temperature interaction proved that the higher the temperature, the less time is required for reduction, whereas the lower the temperature, the longer the treatment needs to be applied for the same results.

Table 1. *Effect of hot water application times on the log reduction[c] (SEM)[d] of each pathogen on lean surfaces.*

	Time (s)		
Pathogen	5	10	15
Salmonella Typhimurium	1.2[b]	1.5[ab]	1.8[a]
	(0.18)	(0.18)	(0.18)
Escherichia coli O157:H7	1.1[b]	1.6[ab]	2.1[a]
	(0.27)	(0.23)	(0.23)

[a,b,] LSMeans within a row without a common letter are significantly different ($P < 0.05$).
[c]Log reduction = (log CFU/cm^2 before treatment)-(log CFU/cm^2 after treatment).
[d]SEM is the standard error of the least squares means.

Table 2. *Interaction of temperature and time of hot water application on the log reduction[e] (SEM)[f] of pathogens on fat surfaces.*

	Temperature and Times								
	66°C			74°C			82°C		
Pathogen	5 (s)	10 (s)	15 (s)	5 (s)	10 (s)	15 (s)	5 (s)	10 (s)	15 (s)
Salmonella Typhimurium	0.7[d] (0.47)	1.4[cd] (0.53)	2.1[bc] (0.47)	1.8[cd] (0.42)	3.5[a] (0.42)	2.6[abc] (0.42)	3.4[a] (0.47)	3.2[ab] (0.47)	2.9[abc] (0.47)
Escherichia coli O157:H7	1.0[c] (0.56)	1.5[bc] (0.63)	2.6[ab] (0.56)	2.1[bc] (0.50)	4.1[a] (0.50)	3.0[ab] (0.50)	3.6[a] (0.56)	4.0[a] (0.56)	3.0[ab] (0.56)

[a,b,c,d] LSMeans within a row without a common letter are significantly different ($P < 0.05$).
[e]Log reduction = (log CFU/cm^2 before treatment)-(log CFU/cm^2 after treatment).
[f]SEM is the standard error of the least squares means.

Table 3 shows the effect of varying temperatures on log reduction on both lean and fat surfaces of beef inside and outside rounds. There were no differences ($P > 0.05$) in log reductions based on time of application (data not shown). For the lean surfaces, the 82°C temperature had a ($P < 0.05$) higher log reduction of both *E. coli* (2.0) and coliforms (2.1) than the 74°C (1.1 and 1.2) or 66°C (0.8 and 0.8) treatments.

Table 4 shows the effects of temperature on color recovery on both lean and fat surfaces. When exposed to hot water, exposed lean surfaces turn from a reddish raw color to a brown cooked color. A major discrepancy of using this method is the muscle color is unable to return to its once red color which means more trimming for producers in turn means loss of money. For the lean surfaces, there were differences ($P < 0.05$) in color for all three temperature treatments. Looking at the 66ºC treatment, the color change was the lowest color recovery value resulting in a moderately pink color; however, the 82ºC color change resulted in a classified moderately pink color. The fat surface did not differ ($P < 0.05$) in regards to the temperature increase. After 24 hours, the three temperatures all returned to a classified white to yellow white, which is an industry accepted fat color.

Table 3. *Effect of hot water temperature application on the log reduction[d] (SEM)[e] of* Escherichia coli (E.coli) *and coliforms on both lean and fat surfaces.*

	Temperature (°C)		
	66	74	82
Lean surfaces			
E. coli	0.8[b]	1.1[b]	2.0[a]
	(0.23)	(0.18)	(0.23)
Coliforms	0.8[b]	1.2[b]	2.1[a]
	(0.25)	(0.20)	(0.24)
Fat surfaces			
E. coli	0.2[c]	1.5[b]	2.7[a]
	(0.31)	(0.24)	(0.31)
Coliforms	0.4[b]	1.7[b]	2.8[a]
	(0.29)	(0.23)	(0.29)

[a,b,c]LSMeans within a row without a common letter are significantly different ($P < 0.05$).
[d]Log reduction = (log CFU/cm^2 before treatment)-(log CFU/cm^2 after treatment).
[e]SEM is the standard error of the least squares means.

Table 4. *Effect of hot water temperature application on recovery color[d] (SEM)[e] values on both lean and fat surfaces.*

	Temperature(°C)		
	66	74	82
Lean surfaces	2.54[c]	3.72[b]	5.27[a]
	(0.29)	(0.28)	(0.29)
Fat surfaces	1.94[c]	2.17[c]	2.03[c]
	(0.28)	(0.29)	(0.28)

[a,b,c]LSMeans within a row without a common letter are significantly different ($P < 0.05$).
[d]Recovery color values were recorded 24 h post treatment.
[d]Recovery color scale for lean: 1 = red, 2 = moderately red, 3 = slightly red, 4 = pink, 5 = moderately pink, 6 = slightly pink, 7= slightly grey or tan, 8 = moderately grey or tan, 9 = grey or tan, 10 = slightly brown, 11 = moderately brown, 12 = brown, 13 = extremely brown.
[d] Recovery color scale for fat:1 = white fat, 2 = yellow white, 3 = yellow, 4 = yellow/brown, 5 = brown fat/greenish.
[e]SEM is the standard error of the least squares means.

Figure 3 observed the accuracy of temperature labels, in comparison to thermocouples, that can sometimes be used in industry to determine if the hot water being used is at the correct temperature. The highest partial change, a circle not completely filled in, was on fat surfaces at 66ºC, and the second highest partial change was on lean surface at 66 ºC. This shows that there were more variation within the temperature labels at lower temperatures than higher, and there were also more variation when the labels were placed on the fat surfaces than the lean surface.

Figure 4 compared the thermocouples to the infrared thermometer reader. Although the industry sometimes uses infrared readers to verify the water temperature, it proved not to be a reliable source of information in this experiment. The readings were lower than the actual thermocouple readings. The difference between the thermocouple and infrared temperatures appears to be greater as the temperature of the water treatment increased.

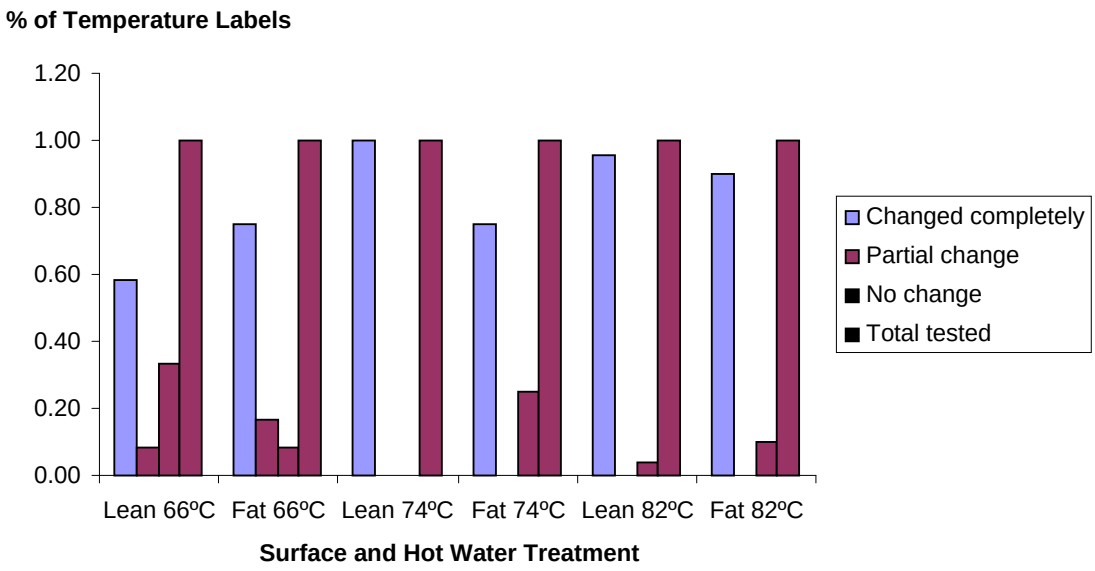

Figure 3. Percent accuracy of temperature labels during hot water treatment of lean and fat surfaces.

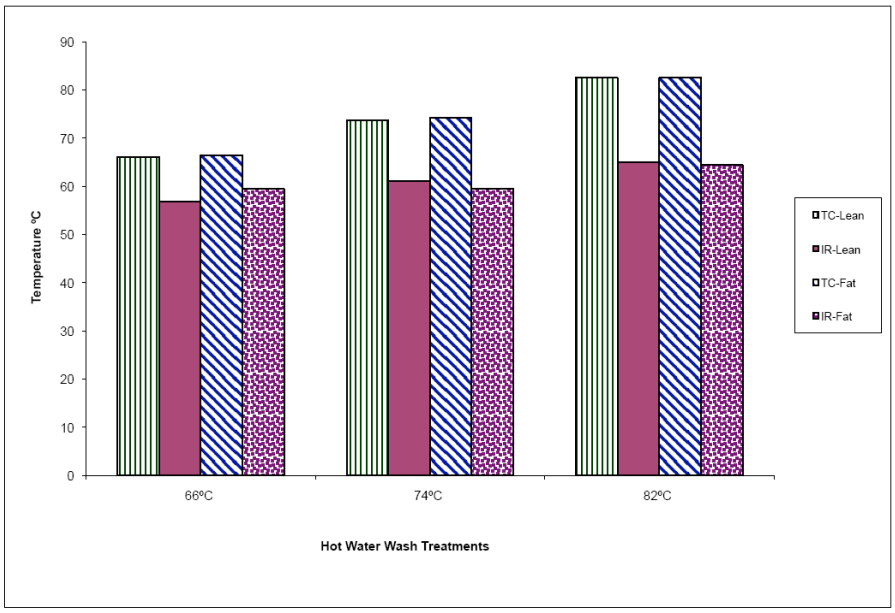

Figure 4. Comparison of thermocouple and infrared thermometer temperatures.

CHAPTER V

DISCUSSION

Lean versus fat surfaces

Observing the differences on the lean surfaces between the two pathogens, were similar. Both had differences with the extreme times (5s and 15 s) by one log difference. If I were a plant that had this thesis to reference, I would have to look at my past tests of generic *E. coli* and coliforms. If they were high, I would definitely use the 15 s, but if they were lower, I believe the 5 s treatment may work.

The outside rounds only temperature statistical difference was 66ºC. The other two temperatures (74ºC and 82ºC) had no statistical differences between the temperatures. In this case, if I had to use either 74ºC or 82ºC, the 5 s would be the logical time to use as to not waste hot water, thus time. If I were to use the 66ºC, I again would look at past tests and go from there. If the bacterial loads need a longer time for a higher kill, then because there is not a statistical difference between 10 s and 15 s, I would use the 10 s.

Regaining normal color

It was reassuring to see the cooked pieces of meat in the 82ºC treatment turn pinkish again the next day. It is known that meat begins to turn brown shades when it reaches higher temperatures. Smith and Graham *(40)* reported the same findings after using 80ºC for 10 s. This lets the producer use the hotter water for the pathogen intervention, yet reduces the amount of trim that will need to be cut off once the product is cooled. The inside rounds tended to come out a moderate pink at 82ºC where the 66ºC

exited the cooler looking redder. Although it is not the brown as the day prior, it was still a color the meat producer could keep. The fat was perfect for this experiment. There was not a difference statistically, or visibly, between the 66 ºC and 82 ºC pieces.

Temperature labels

Temperature labels are sometimes used in industry but from the data gathered in this experiment, they are not that reliable. There was a lot of variation at the lower temperatures than the higher temperatures. Partial changes could have been due to the pin insertion itself by placing it on the meat, causing the dot to change color before getting into the cabinet. Although in theory it is a good concept, if the temperature label is made properly and can withstand the temperatures without falling apart from the water pressure or changing colors during pinning them to the meat for use, then a reassessment may need to be made. Until then, thermocouples would be the most accurate readings.

Infrared thermometers

The infrared thermometer read lower than the actual temperature. Once the higher temperature treatments were being administered, it was harder to get accurate measurements due to the steam that was being produced. Because of this, when reporting the readings if an infrared thermometer is used then a plant may not meet their requirements, or they may overcook the product thinking the water is running cold. Again, my recommendation would be to use a thermocouple to get the readings. Although easy to use, infrared thermometers are not meant to work with this kind of project.

CHAPTER VI

CONCLUSIONS

These data have shown that depending on the initial data load entering the plant, this intervention, would be a good process in someone's HACCP plan. Although at higher temperature applications the meat may have a cooked appearance, this is proven that within 24 hours, the meat will once again rebloom and return to its reddish color. Although the lower temperatures (66ºC) may require longer amounts of time to achieve the same pathogen reduction as the higher temperatures, it still has a log reduction of 2 logs at 15 seconds for both pathogens tested. This is a good log reduction for plants that have low levels of pathogen contamination. For the plants with higher levels of contamination, the 74ºC temperature application may be the treatment of choice. It proved to have a log pathogen reduction of 3.5 and 4.1 for *Salmonella* and *E. coli* O157:H7 respectively. This treatment should ensure a higher decrease at a lower cost to the producer because they will not have to heat the water to hotter temperatures or have more trim from any grey color of the meat that didn't return to its natural color.

Overall, this is a very good process that can be employed easily and can be used as a hurdle intervention to reduce both non-pathogenic and pathogenic bacteria that may be present due to contamination during processing.

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VITA

Melissa Ann Davidson received her Bachelor of Science degree in poultry science from Texas A&M University in College Station in 2001. She entered the poultry science program at the University of Arkansas in May of 2004 where she received her Master of Science degree. Her research interests include food science and keeping the food supply safe from pathogens in all food sources.

Ms. Davidson may be reached at 348 Kleberg College Station, TX 77843-2471.